MODIFIED GRAM STAINS

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The Gram stain continues to be an ever fruitful subject for experimentation. The literature reveals a variety of methods, many of which have .proved valuable. The Society of American Bacteriologists, through the reports of its committee, has offered 2 methods for consideration which have been widely used.[1] More recently, however, 2 important modifications of the Gram stain have been published. The first by Atkins [2] proposes the use of aniline sulphate in place of aniline, and a stronger iodine solution, to which sodium hydroxide has been added. The other method is that described by Burke.[3] Here the chief points of interest are the use of: a 1% aqueous solution of dye, sodium bicarbonate, and acetone as a decolorizer. Both Atkins' and Burke's methods have yielded satisfactory results in our hands, and it would be difficult to enter into a discussion of the relative merits of these methods without previous exhaustive investigation. Suffice it to say that we have taken the liberty of combining some of the principal features of each of these methods in an attempt to economize time and expense without sacrificing the quality of the results. Before advancing the reasons for choice we present the method in detail step by step as used by us.

1. Air dry a thinly spread film and fix with least amount of heat necessary to kill the organisms and fix them to the slide.

2. Flood slide with dye solution. This is prepared by previously mixing in a beaker about 30 drops of a 1% aqueous solution of methyl violet 6 B (Coleman and Bell) with 8 drops of a 5% solution of sodium bicarbonate. Allow the mixture to remain on slide 5 minutes or more.

3. Flush off the excess stain with the iodine solution and cover with fresh iodine solution for 2 minutes or longer. The iodine solution consists of 2 gm. iodine dissolved in 10 c c normal sodium hydroxide solution, to which is then added 90 c c of water.

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[1] Jour. Bacteriol., 1919, 4, p. 107.
[2] Ibid., 1920, 5, p. 321.
[3] Ibid., 1922, 7, p. 159.

4. Drain off the excess iodine solution, without blotting (no water being used), but the film is not permitted to become dry.

5. Add acetone (100%) drop by drop until no color is seen in the drippings from the slide, which is slightly tilted. This usually requires less than 10 seconds and should be reduced to a minimum.

6. Air dry the slide.

7. Counterstain for 10-30 seconds with 0.1% aqueous solution of basic fuchsin.

8. Wash off excess stain by short exposure to tap water and air dry. If slide is not clear immersion in xylol has been recommended.

The advantages from such a procedure are considered separately in the papers of Atkins and Burke and may be briefly enumerated as follows:

In step 2 a 1% aqueous solution of violet (either methyl 6 B, crystal or gentian violet) is easily prepared and has relatively good keeping qualities. Sodium bicarbonate neutralizes acidity and improves the intensity of the stain in the gram-positive organisms. We have found that the more intimate mixture of sodium bicarbonate and dye solution prior to application yields somewhat better results than when these are used separately. A stock solution of dye and bicarbonate cannot be made up since deterioration sets in rapidly. Fortunately there is no danger of overstaining provided that actual drying does not occur. This does not apply to the counterstain.

In step 3 of this iodine formula, sodium hydroxide is used to dissolve the iodine. This is not only economical, but also yields free hydroxyl-ions which may aid in intensifying the stain.

In steps 4, 5 and 8 it will be noted that we do not use blotting paper at any stage in the procedure because it not only appears to be superfluous but may frequently leave fibers on the slide, which are undesirable.

In step 5 acetone has proved to be more satisfactory than either 95% or absolute alcohol and is also more economical as regards expense and quantity.

In step 7 we prefer fuchsin as a counterstain because it is bright and gives an excellent contrast.

The Gram stain described has been used with particularly gratifying results in connection with our studies on the therapeutic effect of Lactobacillus acidophilus.[4] The daily routine required the staining of Lacto-

[4] Kopeloff, Nicholas and Cheney, C. O.: Proc. Soc. Exper. Biol. Med., 1922, 19, pp. 372-373. Jour. Am. Med. Assn., 1922, 79, p. 609.

bacillus acidophilus in milk. Using the Sterling modification of the Gram stain with alcohol as a decolorizing agent, the débris was dark purple which made it somewhat difficult to identify individual bacilli. However, with the procedure here outlined the débris is pale pink, which causes the gram-positive bacilli to stand out sharply. Furthermore, the gram-negative débris does not obscure the presence of gram-negative organisms as gram-positive débris is likely to do. Again the routine examination of feces in these studies [4] was attended with the usual difficulties when the Sterling modification of the Gram stain was employed. The microscopic examinations therefore were greatly facilitated by the present procedure, which not only rendered the débris gram-negative but yielded a sharper differentiation between gram-positive and gram-negative organisms.

In order to widen the scope of its application a number of common pathogenic and saprophytic bacteria were stained by this method. It was found that the organisms were stained in a way that left little to be desired with regard to the Gram phenomenon or morphology. Particular attention may be directed to the clear cut results obtained with gonococcus and the diphtheric bacillus which ordinarily present considerable difficulty in Gram staining.

SUMMARY

A method of Gram staining has been outlined which is based on the results obtained by the use of modifications devised by Burke [3] and Atkins.[2]

The method has yielded particularly good results in staining milk slides for Lactobacillus acidophilus and in staining fecal specimens. By this method, the gonococcus and the diphtheric bacillus are well differentiated and more easily identified than by the older methods. The same was found to be true for a number of common pathogenic and saprophytic bacteria.